

GIJZEN
Serial No. 08/939,905

numbered by assigning +1 to the first base of the ATG start codon; amino acids are numbered by assigning +1 to the N-terminal Gln residue after cleavage of the putative signal sequence. The N-terminal signal sequence, the region of the active site, and the heme-binding domain are underlined. The numerals I, II and III placed directly above single nucleotide gaps in the sequence indicate the three intron splice positions. The target site and direction of five different PCR primers are shown with dotted lines above the nucleotide sequence. An asterisk (*) marks the translation stop codon.

Please replace the paragraph beginning at page 9, line 14, with the following rewritten paragraph:

Figure 2 is the genomic DNA sequence of the Soybean seed coat peroxidase (commencing at nucleotide 1342 of SEQ ID NO:2).

Please replace the paragraph beginning at page 9, line 16, with the following rewritten paragraph:

Figure 3 is a comparison of soybean seed coat peroxidase with other closely related plant peroxidases. The GenBank accession numbers are provided next to the name of the plant

from which the peroxidase was isolated. The accession number for the soybean sequence is L78163. (A) A comparison of the nucleic acids sequences; (B) A comparison of the amino acid sequences (L78163 nucleotide sequence SEQ ID NO:1; L78163 amino acid sequence SEQ ID NO:1, U41657 nucleotide sequence SEQ ID NO:10; U41657 amino acid sequence SEQ ID NO:15; X90693 nucleotide sequence SEQ ID NO:11; X90693 amino acid sequence SEQ ID NO:16; X90694 nucleotide sequence SEQ ID NO:12; X90694 amino acid sequence SEQ ID NO:17; L36156 nucleotide sequence SEQ ID NO:13; L36156 amino acid sequence SEQ ID NO:18; X90692 nucleotide sequence SEQ ID NO:14; X90692 amino acid sequence SEQ ID NO:19).

Please replace the paragraph beginning at page 10, line 8, with the following rewritten paragraph:

Figure 5 exhibits the structure of the *Ep* Locus. A 17 kb fragment including the *Ep* locus is illustrated schematically. A 3.3 kb portion of the gene is enlarged and exons and introns are represented by shaded and open boxes, respectively. The final enlargement of the 5' region shows the location and DNA sequence around the 87 bp deletion occurring in the *ep* allele of soybean line OX312. Nucleotides are numbered by assigning +1 to the

GIJZEN
Serial No. 08/939,905

first base of the ATG start codon (OX347(Ep) sequence defined by nucleotides 1513-1621 of SEQ ID NO:2; OX342(ep) sequence defined by SEQ ID NO:20 (nucleotides 1513-1624 of SEQ ID NO:2 but with deletion of nucleotides 1524-1610).

Please replace the paragraph beginning at page 27, line 15, with the following rewritten paragraph:

PCR amplifications contained 1 ng template DNA, 5 pmol each primer, 1.5 mM MgCl₂, 0.15 mM deoxynucleotide triphosphates mix, 10 mM Tris-HCl, 50 mM KCl, pH 8.3, and 1 unit of Taq polymerase (Gibco BRL) in a total volume of 25 μL. Reactions were performed in a Perkin-Elmer 480 thermal cycler. After an initial 2 min denaturation at 94°C, there were 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 52°C, and 2 min extension at 72°C. A final 7 min extension at 72°C completed the program. The following primers were used for PCR analysis

of genomic DNA:

prx2+	CTTCCAAATATCAACTCAAT	(SEQ ID NO:4)
prx6-	TAAAGTTGGAAAAGAAAGTA	(SEQ ID NO:5)
prx9	ATGCATGCAGGTTTTCACT	(SEQ ID NO:6)
prx10-	TTGCTCGCTTCTATTGTAT	(SEQ ID NO:7)
prx12+	TCTTCGATGCTTCTTCACC	(SEQ ID NO:8)
prx29+	CATAAACAAATACGTACGTGAT	(SEQ ID NO:9)

Please replace the paragraph beginning at page 30, line 12,

with the following rewritten paragraph:

The genomic sequence matched the cDNA sequence except for three introns encoded within the gene. The genomic sequence also revealed two additional translation start codons, beginning one bp and 10 bp upstream from the 5' end of the longest cDNA transcript isolate. Figure 1 (SEQ ID NO:1) shows the deduced cDNA sequence. The open reading frame of 1056 bp encodes a 352 amino acid protein of 38,106 Da. A heme-binding domain, a peroxidase active site signature sequence, and seven potential N-glycosylation sites were identified from the deduced amino acid sequence. The first 26 amino acid residues conform to a membrane spanning domain. Cleavage of this putative signal sequence releases a mature protein of 326 residues with a mass of 35,377 Da and an estimated pI of 4.4.

Please replace the paragraph beginning at page 31, line 11,

with the following rewritten paragraph:

Figure 3 (SEQ ID Nos:10-19) illustrates the relationship between the soybean seed coat peroxidase and other selected plant peroxidases. The soybean sequence is most closely related to four peroxidase cDNAs isolated from alfalfa, (see Figure 3)

sharing from 65 to 67% identity at the amino acid level with the alfalfa proteins (X90693, X90694, X90692, el-Turk et al 1996; L36156, Abrahams et al 1994). When compared with other plant peroxidases, soybean seed coat peroxidase exhibits from 60 to 65% identity with poplar (D30653 and D30652, Osakabe et al 1994) and flax (L0554, Omann and Tyson 1995); 50 to 60% identity with horseradish (M37156, Fujiyama et al. 1988), tobacco (D11396, Osakabe et al 1993), and cucumber (M91373, Rasmussen et al. 1992); and 49% identity with barley (L36093, Scott-Craig et al. 1994), wheat (X85228, Baga et al 1995) and tobacco (L02124, Diaz-De-Leon et al 1993) peroxidases.

Please replace the paragraph beginning at page 33, line 10,
with the following rewritten paragraph:

Primers were designed from the DNA sequence to compare *EpEp* and *epep* genotypes by PCR analysis. Figure 6 shows PCR amplification products from four different primer combinations using OX312 (*epep*) and OX347 (*EpEp*) genomic DNA as template. The primer annealing site for *prx29+* begins 182 bp upstream from the ATG start codon; the remaining primer sites are shown in Figure 1. Amplification with primers *prx2+* and *prx6-*, and with *prx12+* and *prx10-* produced the expected products of 1.9 kb and 860 bp,

GIJZEN
Serial No. 08/939,905

respectively, regardless of the *Ep/ep* genotype of the template DNA. However, PCR amplification with primers prx9+ and prx10-, and with prx29+ and prx10- generated the expected products only when template DNA was from plants carrying the dominant *Ep* allele. When template DNA was from an *epep* genotype, no product was detected using primers prx9+ and prx10- and a smaller product was amplified with primers prx29+ and prx10-. The products resulting from amplification of OX312 or OX347 template DNA with primers prx29+ and prx10- were directly sequenced and compared. The polymorphism is due to an 87 bp deletion occurring within this DNA fragment in OX312 plants, as shown in Figure 5 (SEQ ID NO:20). This deletion begins nine bp upstream from the translation start codon and includes 78 bp of sequence at the 5' end of the open reading frame, including the prx9 + primer annealing site.

Please replace the paragraph beginning at page 35, line 3, with the following rewritten paragraph:

The seed coat peroxidase mRNA levels were determined by hybridizing RNA gel blots with radio labelled cDNA probe. Figure 9 illustrates the transcript abundance in various tissues of *epep* and *EpEp* plants. The mRNA accumulated to high levels in

seed coat tissues of *EpEp* plants, especially in the later stages development when whole seed fresh weight exceeded 50 mg. Low levels of transcript could also be detected in root tissues but not in the flower, embryo, pod or leaf. The transcript could also be detected in seed coat and root tissues *epep* plants but in drastically reduced amounts compared to the *EpEp* genotype. The reduced amounts of peroxidase mRNA present in seed coats of *epep* plants indicates that the transcriptional process and/or the stability of the resulting mRNA is severely affected. The *Ep* gene has a TATA box and a 5' cap signal beginning 47 bp and 15 bp, respectively, upstream from the translation start codon. The 87 bp deletion in the *ep* allele extends into the 5' cap signal and therefore could interfere with transcript processing. Regardless, any resulting transcript will not be properly translated since the AUG initiation codon and the entire amino-terminal signal sequence is deleted from the *ep* allele. Not wishing to be bound by theory, the lack of peroxidase accumulation in seed coats of *epep* plants appears to be due to at least two factors, greatly reduced transcript levels and ineffective translation, resulting from mutation of the structural gene encoding the enzyme. In summary, the results indicate that the *Ep* gene regulatory elements can drive high